Use of Ligase Chain Reaction with Urine versus Cervical Culture for Detection of *Chlamydia trachomatis* in an Asymptomatic Military Population of Pregnant and Nonpregnant Females Attending Papanicolaou Smear Clinics

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Ligase chain reaction (LCR) (Abbott Laboratories, Abbott Park, Ill.) with first-catch urine specimens was used to detect *Chlamydia trachomatis* infections in 465 asymptomatic military women attending clinics for routine Papanicolaou smear tests. Results were compared to results of cervical culture to determine the sensitivity of the urine LCR and the possible presence of inhibitors of amplification in pregnant and nonpregnant women. Discrepant results for LCR and culture were resolved by direct fluorescent antibody staining of culture sediments, two different PCR assays, and LCR for the outer membrane protein 1 gene. The prevalence of *Chlamydia* in specimens by urine LCR was 7.3% compared to 5% by culture. For 434 women with matching specimens, there were 11 more specimens positive by LCR than were positive by culture, of which all but one were determined to be true positives. There were four culture-positive, LCR-negative specimens, all from nonpregnant women. The sensitivity, specificity, and positive and negative predictive values of urine LCR after discrepant results were resolved were 88.6, 99.7, 96.9, and 99.0%, respectively. The sensitivity of culture was 71.4%. From the 148 pregnant women (prevalence by LCR, 6.8%), there were no patients who were cervical culture positive and urine LCR negative to indicate the presence in pregnant women of inhibitors of LCR. Additionally, a subset of 55 of the LCR-negative frozen urine specimens from pregnant women that had been previously processed in LCR buffer were inoculated with 5 cell culture inclusion forming units of *C. trachomatis* each and retested by LCR; all tested positive, indicating the absence of inhibitors of LCR in urine from these pregnant women. The use of LCR testing of urine specimens from asymptomatic women, whether pregnant or not, offers a sensitive and easy method to detect *C. trachomatis* infection in women.

Approximately 4 million *Chlamydia trachomatis* urogenital infections occur in the United States annually, and more than 50 million cases occur worldwide (7, 28). Unfortunately, symptoms are often mild or absent among infected men and women, leaving a large reservoir of infected persons to continue transmission to new sex partners (29). Chlamydial infections occur primarily among young sexually active persons. A high prevalence is common to all socioeconomic groups and may range from 5 to 20% in various groups of young adults (32, 33). Because of the high probability of progression of asymptomatic disease to serious sequelae, it has been recommended that individuals at risk for chlamydial infections be screened, especially women who are vulnerable to the serious consequences of genital infections, such as pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (7, 11). Urine can now be used to detect chlamydial infections in women by ligase chain reaction (LCR) (2, 8, 14, 20, 31, 34), which with its easily obtained specimen is a cost-effective method for screening programs for asymptomatic women (16). Because asymptomatic military populations have not been studied widely with

regard to chlamydial infections (4, 6, 10, 21, 26, 27) and because the sensitivity of the urine LCR assay has been reported to be low for samples from pregnant women due to the presence of inhibitors to amplification (18), we compared urine LCR to cervical culture for the detection of *C. trachomatis* in asymptomatic women attending clinics for routine Papanicolaou (PAP) smear tests.

MATERIALS AND METHODS

Populations and specimens. Military women ($n = 480$) attending clinics for a routine PAP smear test volunteered for a study to compare urine LCR tests to cervical cultures for the detection of *C. trachomatis* infections. The volunteer rate of the women approached by the civilian research nurse was 71%. The study was approved by the Institutional Review Boards of The Johns Hopkins University, the U.S. Army Medical Research Material Command, Fort Detrick, Fredrick, Md., and Womack Army Medical Center, Fort Bragg, N.C. Of 480 women enrolled, 465 provided a urine specimen. All subjects completed a questionnaire for demographic information and behavioral risk factors for sexually transmitted diseases. The data instrument was a one-page, two-sided scanable bubble form (Scanntron Corporation, Tustin, Calif.). During the pelvic examination, an endocervical swab was obtained by the attending clinician at the PAP smear clinic, who recorded clinical signs and symptoms on the data form. Culture swabs were placed into 2-sucrose-phosphate chlamydia transport medium. Commercial transport medium was replaced with in-house transport medium after 1 month of the study due to some toxicity of the former to tissue culture cells. Specimens were stored appropriately (4°C for urine specimens and −70°C for cultures) until shipping of the urine specimens at 4°C and cultures at −70°C. Shipments were made to ensure arrival at the laboratory within 4 days of collection. All specimens, consent forms, and data forms were shipped to Johns Hopkins Chlamydia Research Laboratory.

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Laboratory procedures. Urine specimens were processed and tested by LCR (Abbott Laboratories, Abbott Park, Ill.) according to the manufacturer's instructions. Briefly, 1 ml of urine was centrifuged at $15,000 \times g$ for 15 min. After the supernatant was removed, 1 ml of urine buffer was added to the pellet and the mixture was vortexed. After being heated at 97°C for 15 min, specimens were cooled and 100 μl of each specimen was added to an LCR unit dose tube. An appropriate chlamydia-positive control was included for the processing steps for each group of specimens. Additionally, two negative controls and two positive calibrator controls supplied by the manufacturer were used for each LCR assay run. After the amplification step in the automated thermocycler, unit dose tubes containing the specimens and controls were transferred to the automated enzyme immunoassay machine for the detection of amplified products. Tubes containing the amplified products were never opened; the automated enzyme immunoassay process sampled tubes by piercing the tops of the unit dose tubes, which prevented amplicon contamination. In order to prevent other sources of contamination, specimens were processed in a designated room separate from the room used to amplify and detect specimens. Gloves were frequently changed and aerosol-barrier pipette tips and dedicated pipettors were used. Strict quality-control measures such as machine maintenance checks, daily cleaning of laboratory areas and equipment with bleach, and area swipe tests to monitor amplicon contamination were employed.

Culture specimens were stored frozen at −70°C for up to 3 days. Cultures were done in 96-well microwell plates in McCoy cells by standard methods (12). Tissue cultures were stained with genus-specific fluorescein-conjugated antibody (Kallested, Chaska, Minn.) and species-specific antibody (Boehringer Mannheim/Syva, San Jose, Calif.). Stained cultures were read for the presence of chlamydial inclusion bodies with an epifluorescence microscope.

Discrepancy analysis was done for any sample with discordant results between culture and LCR. A sample that was positive by culture and negative by LCR was considered to be a true positive, but the discrepancy was investigated for the presence of inhibitors to amplification by LCR. The urine LCR was repeated from the originally processed specimen and repeated again after diluting the processed specimen 1:10 in urine LCR buffer to check for the presence of inhibitors in the specimen. (Dilution has been shown to sometimes decrease the concentration of the inhibitor enough to allow a true-positive specimen to be amplified.) Additionally, PCR (Roche Diagnostic Systems, Branchberg, N.J.) was done on an archived aliquot of frozen urine and another LCR was done for a different DNA target, the outer membrane protein 1 (OMP-1) gene. For specimens that were positive by LCR and negative by culture, the culture specimen transport sediment was stained by direct fluorescent antibody (DFA) (Boehringer Mannheim/Syva) for chlamydial elementary bodies. PCR also was done on the specimens from the culture transport vials. In addition, PCR was done on the archived urine and an LCR for the OMP-1 gene was done on the previously processed (buffered) urine specimen. Specimens that were positive by one or more of the ancillary tests were considered true positives. An LCR-positive urine specimen which could not be confirmed by another test was considered to be a false positive.

Testing of urine specimens from pregnant women. A subset of all available (n = 55) previously processed (buffered) LCR-negative urine specimens that were from pregnant women were inoculated with 5 inclusion forming units of *C. trachomatis* and retested by LCR to check for the presence of inhibitors. Additionally, 65 archived LCR-negative unprocessed urine specimens that were available from pregnant women were tested by a research internal control assay to evaluate the presence of inhibitors (9). This assay tested for the ability to amplify an extraneous sequence of DNA which was added as an internal control to the specimen. The assay contained primers for the extraneous DNA internal control as well as the primers for the organism of interest. A positive amplification of the internal control indicated that the specimen contained no inhibitors to the amplification process, while a negative result indicated that the specimen contained something which inhibited the amplification process.

Data analysis. The data from the questionnaire forms were scanned into a data set (D-base III Plus; Ashton Tate, Borland International, Spring Valley, Calif.), and LCR results, demographics, and risk factor information were analyzed by the chi-square test, Fisher's tests of exactness, and univariate analysis (Intercooled Stata, version 4.0; Stata Corporation, College Station, Tex.).

RESULTS

Patient characteristics. Among the 480 women enrolled, only 1 woman had reported mild symptoms and the remainder were asymptomatic. Approximately half (55.2%) were 25 years or younger, and 50.8% were African-American. Over 90% were enlisted personnel, 98.3% reported vaginal sex, 11.3% had a new sex partner in the previous 90 days, 15.2% had more than one sex partner in the previous 90 days, 88.5% reported inconsistent condom use, and 30.8% were pregnant (Table 1). Reasons for clinic visit, clinical presentation, and sexual risk history are presented in Table 1. Of the 465 women who provided a urine specimen, the overall prevalence for chla-

TABLE 1. Characteristics of military women screened for *C. trachomatis* at Fort Bragg, N.C.

Variable	No. of women (n = 480)	%
Median age, yr (range)[a]	25 (19–47)	
Ethnicity[b]		
White	181	37.7
African-American	244	50.8
Other (American Indian, Alaskan, or Asian Pacific)	52	10.8
Military category[c]		
Enlisted personnel	439	91.5
Officer	36	7.5
Reason for clinic visit[c]		
Sex partner of infected individual	1	0.2
Complaint of symptoms	1	0.2
Screening	470	97.8
Other	4	0.8
Clinical presentation[d]		
Mucopus	1	0.2
Cervicitis	5	1.0
Ectopy	5	1.0
Cervical motion tenderness	3	0.6
Friability	39	8.1
Pregnant	148	30.8
Normal exam	348	72.5
Sexual history (past 90 days)		
More than one sex partner	73	15.2
New sex partner	54	11.3
Consistent condom use	51	10.6
Inconsistent condom use	425	88.5
Previous diagnosis[e]		
N. gonorrhoeae	20	4.2
C. trachomatis	86	17.9
Syphilis	5	1.0
Trichomonas	41	8.5
None	348	72.5
Chlamydia-positive urine LCR[f]	34	7.3
Chlamydia-positive culture[g]	24	5.3

[a] Data missing for eight women (1.7%).
[b] Data missing for three women (0.6%).
[c] Data missing for five women (1.0%).
[d] Data missing for 27 women (5.6%).
[e] Data missing for two women (0.4%).
[f] Urine specimen for LCR missing for 15 women.
[g] Cervical specimens for culture missing for 21 women and 10 toxic specimens collected.

mydia infection by LCR was 7.3%. The prevalences of infection for other categories based on LCR included 11.0% for women ≤25 years of age, 8.9% for African-American women, and 6.8% for pregnant women. By risk category the prevalences were 15.1% for those with a new sex partner in the previous 90 days, 10.3% for those with more than one sex partner in the previous 90 days, 7.5% for those with inconsistent condom use, 7.4% for those reporting vaginal sex, and 3.6% for those with a prior chlamydial infection.

In univariate analysis only young age (≤25 years) (odds ratio [OR], 4.23; 95% confidence interval [CI], 1.72 to 10.43) and a new sex partner (OR, 2.61; 95% CI, 1.11 to 6.1) were predictors of chlamydial infection (Table 2). However, when we controlled for age, a new sex partner was no longer significant.

Comparison of urine LCR to cervical culture. Of the 465 women, 31 women did not have matched culture specimen results. Ten specimens were toxic for tissue culture and no cervical cultures were collected from 21 women, leaving 434 matched specimens for comparison. After the use of the commercial chlamydia transport buffer was stopped and the in-

TABLE 2. Univariate analysis of results relative to factors associated with positive urine LCRs for
military women attending PAP smear clinics

Factor[a]	% with a positive LCR		OR (95% CI)	P
	Factor absent	Factor present		
Age ≤25 yr (254)	2.8	11.0	4.2 (1.72, 10.43)	0.002
African-American (233)	6.8	8.6	1.3 (0.61, 2.7)	0.501
Pregnant (142)	7.4	7.0	0.94 (0.44, 2.03)	0.882
Normal pelvic exam (275)	12.5	6.6	0.49 (0.18, 1.31)	0.154
Prior diagnosis of STD[b] (127)	8.3	3.9	0.45 (0.17, 1.2)	0.110
Having had more than one sex partner in last 90 days (68)	6.8	10.3	1.6 (1.53, 3.73)	0.316
Having had a new sex partner in last 90 days (53)	6.4	15.1	2.6 (1.11, 6.10)	0.027[c]

[a] Numbers in parentheses represent numbers of individuals with the factor present ($n = 465$).
[b] STD, sexually transmitted disease (chlamydia, gonorrhea, syphilis, or trichomonas).
[c] A new sex partner was not significant when we controlled for age.

house 2-sucrose-phosphate medium was used, no further specimens toxic to tissue culture were observed. Among the 31 specimens without matched results, there were two LCR-positive urine specimens for which a matching cervical culture was not collected.

From the 434 matched specimens, 32 (7.4%) were LCR positive, of which 31 (7.3%) were confirmed as true positives (Table 3). There were 21 LCR-positive, culture-positive specimens. Four patients had urine-LCR-negative, cervical-culture-positive specimens. Discrepancy analysis of these LCR-negative, culture-positive specimens demonstrated that one was positive in the repeat LCR assay and was OMP-1 LCR positive, one had a negative value which was close to the cutoff value for a positive result and was PCR positive when the archived frozen urine was tested, one had a culture transport specimen that was PCR positive, and the results of one could not be confirmed by any of the ancillary tests, including repeat culture. The initial LCR-negative results from these four urine specimens were all considered to be false negatives.

There were 11 specimens that were LCR positive and culture negative, 10 of which could be confirmed as true-positive specimens (Table 4). Five were DFA positive, six were urine PCR positive, seven were culture PCR positive, and eight were OMP-1 LCR positive. Thus, all but one of these LCR-positive specimens were confirmed as true positives by at least one or more additional assays. After resolution of the discrepant results, the sensitivity, specificity, and positive and negative predictive values of urine LCR were 88.6, 99.7, 96.9, and 99.0%, respectively (Table 3), and the sensitivity of culture was 71.4%.

LCR of urine of pregnant women. There were 148 urine specimens from pregnant women. The prevalence of chlamydia infection by LCR for the pregnant women was 6.8%, and that for the nonpregnant women was 7.8%. There were no culture-positive, LCR-negative results from pregnant women which could have indicated the presence of LCR inhibitors. All four of the culture-positive, LCR-negative specimens were from women who were not pregnant. In addition, a subset of 55 LCR-negative urine specimens, previously processed in LCR buffer and frozen, which were from pregnant women and were inoculated with chlamydia and retested by LCR were all LCR positive, indicating the lack of inhibitors. Of the 65 available archived urine specimens from pregnant women which were LCR negative and tested in the internal control assay, there were 3 (4.6%) that exhibited inhibition based on a negative value for amplification of the internal control.

DISCUSSION

Chlamydia infections were of a higher prevalence than expected from these asymptomatic military women attending a clinic for a routine PAP smear test. An LCR prevalence of 7.3% underscores the necessity for the recommendation to screen all sexually active young women when they are attending a routine health care clinic (7). The high prevalence of 11.0% for those ≤25 years of age confirm the result of studies of others that young age is a significant risk factor for chlamydial infections (13, 17, 22). These results indicate the need

TABLE 3. Comparison of urine LCR to cervical culture for
C. trachomatis in military women attending
PAP smear clinics

Test	Result	No. of women[a] with test result (%)	Resolved patient infection status[b]	
			Positive	Negative
Cervical culture	Positive	25 (5.8)	25	0
	Negative	409 (94.2)	10	399
Urine LCR[c]	Positive	32 (7.4)	31	1
	Negative	402 (92.6)	4	398

[a] Four hundred eighty women enrolled; 434 had matching specimens.
[b] Resolved patient infection status was defined as the resolved status of a patient with a positive culture or with a urine specimen positive by two tests (LCR, DFA staining, PCR OMP-1 gene, PCR plasmid gene, and LCR OMP-1).
[c] After discrepant results were resolved, sensitivity was 88.6%, specificity was 99.7%, and positive and negative predictive values were 96.9 and 99.0%, respectively (sensitivity of culture, 71.4%).

TABLE 4. Resolution of urine-LCR-positive and cervical-culture-negative discrepant results for *C. trachomatis* in military women attending PAP smear clinics ($n = 11$)

Laboratory no.	Test result					Status
	LCR (urine)	DFA[a]	PCR (urine)	PCR (cervix)	LCR for OMP-1 (urine)	
1264	+	−	−	−	+	Confirmed
2407	+	+	+	+	+	Confirmed
3197	+	+	+	+	+	Confirmed
3659	+	−	−	+	−	Confirmed
3891	+	−	−	−	+	Confirmed
5560	+	+	−	+	+	Confirmed
5570	+	−	−	−	−	Unconfirmed
6082	+	−	+	+	+	Confirmed
6280	+	+	+	+	−	Confirmed
6966	+	+	+	+	+	Confirmed
8016	+	−	+	−	+	Confirmed

[a] DFA staining of culture transport vial specimen.

for an ongoing chlamydial control program for such female military personnel as those enrolled in this study. This population demonstrated a high degree of sexual behaviors placing them at risk for sexually transmitted diseases, with 98% being sexually active, 15% having more than one partner, 11% having a new partner in the last 90 days, and 88% using condoms inconsistently. All of these behaviors have been shown by others to be predictive of chlamydial infection (1, 22–24, 36). In the univariate analysis for this study, both young age (prevalence, 11.0%) and having had a new partner (prevalence, 15.1%) reached statistical significance. However, when we controlled for age, a new sex partner was not significant. Young age (≥25 years), which is an easily determined risk factor and which is a nonthreatening question for those women who may be reticent to answer questions about their sexual behavior, appears to be an excellent predictor of chlamydia infection and can be recommended for deciding who should be screened in clinical or outreach situations (13, 17).

Urine LCR performed well in this study of asymptomatic women, with a sensitivity of 88.6%, which is similar to that demonstrated by others for asymptomatic women (87.5%) (2). Compared to cervical culture, which had a sensitivity of 71.4%, LCR detected more infected women. Many reasons can account for the lower culture sensitivity. Not only can the cold chain of transport be interrupted, but the quality of the transport medium is important as well. Initially, a commercially available transport medium was used in this study, which resulted in many (10) toxic tissue culture results. Quality-control assays of the remaining lot of uninoculated transport medium demonstrated that it was toxic to cells in tissue culture. After switching to the use of our own transport medium, which is quality controlled in tissue culture, we observed no further toxicity.

Additionally, the quality of the endocervical specimen, as measured by the presence of columnar epithelial cells, has been shown to play a significant role in the numbers of positive specimens (19, 37). In another study of family-planning clinics in Baltimore, Md., clinicians obtained adequate specimens only 72.3% of the time (37). Thus, inadequate cervical swab specimens could have contributed to the lower sensitivity of culture in our study. Other studies have demonstrated higher sensitivities for urine LCR than cervical culture (2, 5, 8, 20, 31, 34). Sensitivities for cervical culture in these studies has ranged from 45.5 to 46.9% to 55.6 to 65.0% (2, 5, 8, 34). Schachter et al. have demonstrated that the sensitivity of culture for *C. trachomatis* may be increased from 67.1% to 74% by adding a urethral swab culture, which could be indicative that some women may be infected only in the urethra and not the cervix (31). This could help explain the higher number of positives found by urine LCR, presumably reflecting infections from both the cervix and the urethra. Because urine is an easy-to-obtain, noninvasive specimen giving accurate results with LCR, it is ideal for screening asymptomatic individuals who may not be presenting for a pelvic exam or for outreach screening programs.

Although our study enrolled only 148 women who were pregnant, we did not observe any indication of inhibitors in urine specimens, as evidenced by the lack of urine-LCR-negative results when the cervical culture was positive. Although there were four such specimens in this study, they were all from nonpregnant women. Another study has reported a significant problem with inhibitors in urine with use of the LCR test; however, the urine specimens were transported at ambient temperatures, which may have influenced the LCR results (18, 25, 30). The spiking experiment in our study did not demonstrate any inhibitors in the 55 LCR-negative, previously frozen urine specimens from pregnant women. It is possible that freezing and thawing of these processed urine specimens reduced or destroyed some LCR inhibitors. Freezing and thawing reduced the inhibition from 19 to 16% in one study (35). Additionally, the experiment which tested the archived urine of 65 pregnant women demonstrated only three (4.6%) inhibited specimens. This value is of the same order of magnitude as that reported by others for inhibition in urine specimens (2.6 and 1.8%) for amplified testing (3, 15). Most investigators now believe that inhibitors to amplification exist for both urine and cervical specimens (3, 15, 35). A combination of heat treatment (95°C for 10 min) and 10-fold dilution of the processed specimen reduced inhibition of PCR from 19 to 4% in one study (35). The pH of the cervical mucosa was partly correlated with inhibitors (35). Decreased inhibition was found at pH values of ≥7.5. The degree to which inhibitors to amplification influence the prevalence detected by LCR and PCR needs to be further studied. Roche Molecular Systems has addressed this problem by incorporating an internal DNA control amplification and detection assay into their new combination PCR assay for *C. trachomatis* and *Neisseria gonorrhoeae*, which will prove to be a great advance in the diagnostic capability of amplification assays. Specimens exhibiting inhibitors can be diluted or heated and their DNA can be extracted, and tests can be repeated. The use of the internal control will give a greater degree of confidence to the validity of a negative amplification result. Consideration of the use of an internal control should be given for amplification tests in the future. The College of American Pathologists now requires examination of a control to assess the presence of inhibitors in all amplification procedures.

In summary, young sexually active women, including those in the military, should be frequently screened for chlamydia infections. Urine LCR offers an easy and sensitive method to accomplish this, especially for women not presenting for a pelvic examination. It is cost-effective in preventing the expensive sequelae of pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (16).

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